Review of the genus *Townesilitus* (Hymenoptera, Braconidae) in Sweden, with description of a new species and a molecular characterization

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The tribe Townesilitini (Braconidae, Euphorinae) includes the genera *Townesilitus*, *Streblocera*, *Marshiella* and *Prochlithrophorus*. In Sweden this tribe is represented by the genera *Townesilitus* and *Streblocera*. This paper explores the taxonomy of the genus *Townesilitus* in Sweden. One new species is described from Sweden, *Townesilitus oelandicus* sp. nov. and the species *T. aemulus* (Ruthe, 1856) is recorded for the first time for Sweden. All five Swedish species, *T. aemulus* (Ruthe, 1856), *T. bicolor* (Wesmael, 1835), *T. deceptor* (Wesmael, 1835), *T. fulviceps* (Ruthe, 1856) and *T. oelandicus*, are diagnosed both morphologically and molecularly. A key for the identification of these species is provided and a phylogenetic tree is presented as well as information on distribution and phenology for all five species occurring in Sweden.

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The tribe Townesilitini Shaw 1985, are a group of rather small hymenopterans diagnosed for instance by having 5-6 segmented maxillari palpi, labial palpi 3 segmented, ventral rim of clypeus with granulated sculpture, eyes bare; first metasomal tergite petiolate; vein RS+M and second submarginal cell absent. The tribe includes the genera *Townesilitus* Haeselbarth & Loan, 1983, *Marshiella* Shaw, 1985, *Streblocera* Westwood, 1833 and *Proclithrophorus* Tobias & Belokobylskij, 1981 (Stigenberg et al. 2015). The genus *Townesilitus* includes ten species worldwide, with five species occurring in Sweden. Here we focus on the five species of *Townesilitus* found in Sweden.

The *Townesilitus* attack adult beetles of the family Chrysomelidae (Loan 1967, Wylie 1983, Ekbom 1990), especially flea beetles of the genus

Phyllotreta. In Europe T. bicolor commonly parasitizes over 15-50% of the adults of *Phyllotreta* species at localities studied (Wylie 1983, Ekbom 1990, 1991, 2010). As flea beetles are important pests of crucifers, especially rapeseed, the effects and impacts have been studied by researchers with agricultural interests. In the middle part of Sweden the most abundant pest species on rape is *Phyllotreta undulata* (Ekbom & Kuusk 2005) (Fig. 1). The second generation in some southern European populations appears at the end of July and can survive until the end of August. The life cycle of T. bicolor starts with a female laying an egg in a newly hatched flea beetle in late summer. When the flea beetle retreats for its overwintering site, the parasitoid larva has already developed to a first instar larvae inside the flea beetle. In spring (end of May), when the flea beetle returns



Figure 1. Species of *Townesilitus* are parasitoids of adult flea beetles, here *Phyllotreta undulata*, a common host of *T. bicolor*.

Släktet *Townesilitus* parasiterar olika arter av jordloppor. Här avbildas den randiga jordloppan *Phyllotreta undulata*, den vanligaste värden för *T. bicolor*. Baggens längd är ca 2 mm.

to the fields after the overwintering, the parasitoid larvae resume its development. Sometime during the shift between spring and summer the parasitoid leaves its host (killing it), pupates in the soil and emerge 2-3 weeks later as an adult parasitic wasp and begin to parasitize the new generation of flea beetles (Ekbom 1990, Ekbom 2010). In Russia (Penza, Voronezh and Orel regions) the braconid larvae hatched and fed on the host for 10-14 days, then formed cocoons on the upper level of the soil where they pupated five days later. Adult wasps emerged after 10-14 days (Pavlov 1960). As in Sweden, larvae that hatched late (September) overwintered in the host and continued development the following spring. In Russia, two generations per year were noted for *T. bicolor* (Pavlov 1960). Haeselbarth (1988) noted that T. bicolor even can have 2-3 generations per year, the third generation is not found in northern European countries. In Sweden, the flea beetle has only one generation a year.

Loan (1967) describes the oviposition behaviour of *T. bicolor* as follows: "the female leaps onto the elytra of the host and, facing the thorax and parallel to the body of the host, immediately inserts the ovipositor into the apical region of the hemocoel". Wylie and Loan (1984) also noted that the two morphologically similar genera Townesilitus and Microctonus have different oviposition behaviours. While Townesilitus females only oviposit in the abdomen, the females of Microctonus stand beside the flea beetle and oviposit in to the head, thorax or abdomen. In the laboratory several Townesilitus females laid eggs in the same host, but only one parasite larva emerged per host (Wylie 1983), suggesting that Townesilitus species are to be solitary parasitoids.

In this paper I reviewed the species occurring in Sweden, based on study of type specimens as well as material available in the collections at the Swedish Museum of Natural History, using morphological and molecular analyses. I describe one new species and present descriptions, diagnoses and a key to the five different species occurring in Sweden.

Materials and methods

Specimens were collected within the Swedish Malaise Trap Project (SMTP) and by the collecting efforts of Dr. Christer Hansson at Lund University. The whole collection of *Townesili*tus as well as type material of T. aemulus and T. fulviceps was studied at the British Museum of Natural History, London (BMNH). Type material of T. bicolor and T. deceptor was loaned from the Royal Institute of Natural sciences, Bruxelles. Number of examined specimens collected in Sweden: T. aemulus: 47, T. bicolor: 25, T. deceptor: 39, T. fulviceps: 1, T. oelandicus: 1. The material in Haeselbarth's collection at Die Zoologische Staatssammlung München (ZSM) was also studied as well as 7 specimens of T. deceptor from Japan $(5\mathfrak{Q}, 2\mathfrak{Q})$. Morphological terminology follows Wharton et al. (1997) and van Achterberg (1993). The phylogenetic analyses were performed with Bayesian inference (MrBayes v3.2.2, Ronquist et al. 2012). All

Bayesian inference analyses were run through the Cipres web portal for Phylogenetic analysis (www.phylo.org). For molecular methods using the mitochondrial marker COI, see Stigenberg et al. (2015). As outgroup we have used Zagryphus nasutus (Ichneumonidae, Tryphoninae) and a more closely related genus, Meteorus sibyllae (Braconidae, Euphorinae). Sequences were assembled, edited and imaged using Geneious version 8.1 created by Biomatters. For storing voucher and DNA sequence data the program Voseg 1.7.3 (Peña and Malm 2012) was used. Sequences are deposited at GenBank under the accession numbers: MF662768-795 with additional sequences also used in the publication by Stigenberg et al. (2015) (KJ591529 to KJ591534, KJ591536, KC213197) and Stigenberg and Ronquist (2011) (HQ264038). Images were taken using three systems; Canon EOS D50 with a MP-E 65 mm lens and stacked using Zerene Stacker software, Dino-Eye Eyepiece camera mounted on Leica M125 stereo microscope, stacked using Adobe Photoshop CS5, an Infinity X digital camera mounted on an Olympus SZX12 stereo microscope and processed using DeltaPix InSight software. Worldwide distribution data was found using Taxapad (Yu et al. 2011). Local Swedish distribution data is displayed using ArcGIS.

Genus Townesilitus Haeselbarth & Loan 1983 Type species: *Microctonus bicolor* Wesmael 1835

Diagnosis: Easily confused with *Microctonus*, the paraphyletic subgenus of *Perilitus* (Stigenberg et al. 2015). Distinguishing characters for *Townesilitus* are the broad clypeus with ventral rim with granulated sculpture (Fig. 2). Length of fore wing 1.5-3.0 mm long. Tip of mandibles not conspicuously twisted, bifid with the lower tooth shorter than upper tooth. Maxillary palpi 5-6 segmented, labial palpi 3 segmented. Eyes bare, occipital carina complete. Thorax moderately stout, about 2.0 times as long as high and 1.5 times as high as broad. Propodeum carinate, with variable intensity but usually with two longitudinal carinae, sometimes dividing the propodeum into dorsal and posterior part. The



Figure 2. Clypeus with ventral rim with granulated sculpture. *T. deceptor*.

Munsköld med typiskt kornig struktur på nederkanten.



Figure 3. Ventral view of the second metasomal tergite (petiole). Illustrating how it is fused. *T. deceptor*.

Den andra metasomala tergiten (petiolus) avbildad underifrån. Bilden illustrerar hur den är ihopfogad undertill.

posterior part of the propodeum more or less excavated/depressed medially. The 1st metasomal tergite (petiole) fused ventrally (Fig. 3), posteriorly reaching about as far back as where the spiracles are situated. Second metasomal tergite dorsally longitudinally striate. No dorope. Metasomal tergites smooth, shining and with some scattered setae. Ovipositor excerted, slightly down curved or S-curved, when concealed in sheaths often appearing straight.

Distribution: Western and Eastern Palearctic, Nearctic, Oriental.

Ecology: Parasitoid of adult flea beetles (Alticini: Chrysomelidae: Coleoptera).

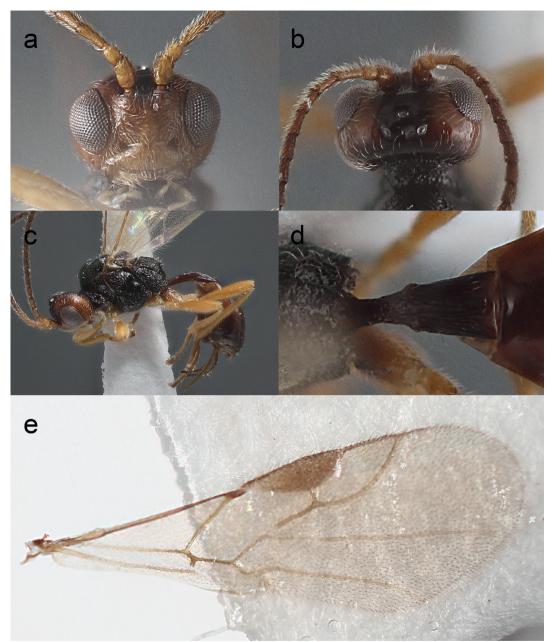


Figure 4. Townesilitus aemulus: -a) head frontal view, -b) head dorsal view, -c) habitus, -d) dorsal view of petiole, -e) fore wing. Figures a-d are specimen JS10_00221, figure e is from specimen Euph_058.

Townesilitus aemulus: – a) ansikte, – b) huvud ovanifrån, – c) habitus, – d) petiolus ovanifrån, – e) framvinge. Figurerna a till d är individ JS10_00221, figur e är från individ Euph_058.

The Swedish species of Townesilitus

Townesilitus aemulus (Ruthe, 1856)

Microctonus aemulus Ruthe, 1856, new comb. by Haeselbarth (1988).

Microctonus punctifrontis Watanabe, 1955, syn. by Belokobylskij (2000).

Redescription

Antennal segments: 19-26. Male antennae densly setate. Head wide, wider than in T. bicolor. Face about twice as wide as high, finegrained – almost matt (Fig. 4a). Clypeus twice as wide as high. Malar space about 0.6 times the basal width of mandible. Ocelli small with rather deep pit between the posterior ocelli (Fig. 4b). Forewing length 2.2-3.0 mm (Fig. 4e). Mesoscutum on the middle lobe setate- punctate, side lobes smooth. Mesopleuron and propodeum quite strongly granulated to reticulate-rugose (Fig. 4c, d). Hind tibia considerably widened to the end, much longer than tarsi. First metasomal tergite 2.0-3.0 times as long as wide, curved (lateral view), the two posterior thirds striated. Ovipositor almost as long as the hind femur, about 0.5 mm. Ovipositor weakly ventrally down curved, the sheath at the tip broadened slightly, spoon-like.

Length: 2.0-2.5 mm, length of type specimen: 2.1 mm.

Diagnosis: *T. aemulus* is very similar to *T. bicolor* but *T. aemulus* generally has more antennal segments, up to 26 segments and also a wider head. Swedish specimens at the NHRS have at most 24 segments. Haeselbarth noted that there were some specimens that were hard to place as *T. aemulus* or *T. bicolor* and I agree.

Distribution: Western Palaearctic and Eastern Palaearctic. In Sweden *T. aemulus* has been found in Northern Sweden (Lapland) and in Southern Sweden (Öland, Gotland and Skåne) (Fig. 5).

Ecology: *T. aemulus* is known in Japan to parasitize on *Psylloides punctifrons* Baly (Watanabe 1955).

Biology: Main flight period in Sweden is in July with some specimens collected at the end of June and beginning of August.

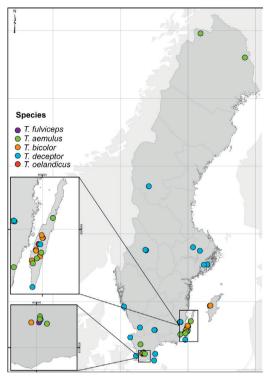


Figure 5. Distribution of the Swedish records of the genus Townesilitus.

Fyndplatser för släktet Townesilitus i Sverige.

Townesilitus bicolor (Wesmael, 1835)

Microctonus bicolor Wesmael, 1835 Microctonus breviradialis Tobias, 1976 syn. by Haeselbarth 1988

Redescription

Antennal segments: 17-22. Head viewed from the front slightly plump, oval narrowed down, usually less wide than *T. aemulus*. From above (dorsally) very variable. Clypeus more than twice as wide as high (♂1.9-2.5). Face about 1.5 times as wide as high (♂1.8-2.1), in the middle convex, quite weak and irregularly wrinkled but stronger than the clypeus (Fig. 6a, b, c). Malar space about twice the width of mandible base

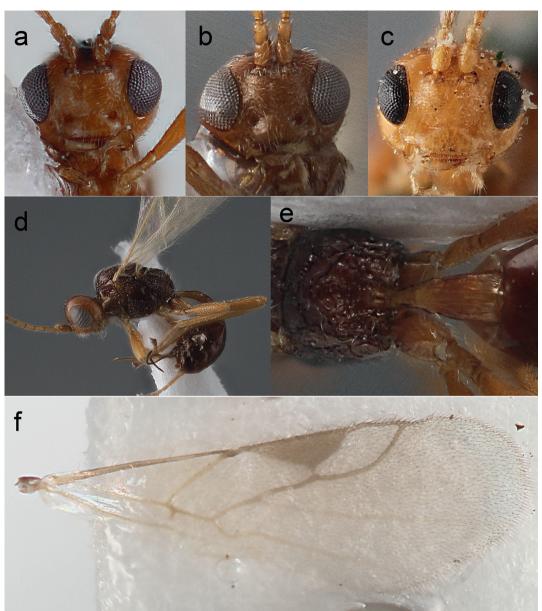


Figure 6. *Townesilitus bicolor*: – a) head frontal view of specimen Euph_134, – b) head frontal view of specimen JS10_00132, – c) head frontal view of specimen Lectotype from IRSB, – d) habitus of specimen JS10_00132, – e) petiole of specimen JS10_00132, – f) fore wing of specimen Euph_045.

Townesilitus bicolor: – a) ansikte av individ Euph_134, – b) ansikte av individ JS10_00132, – c) ansikte av lectotypen från IRSB, – d) habitus av individ JS10_00132, – e) petiolus av individ JS10_00132, – f) framvinge av individ Euph_045.

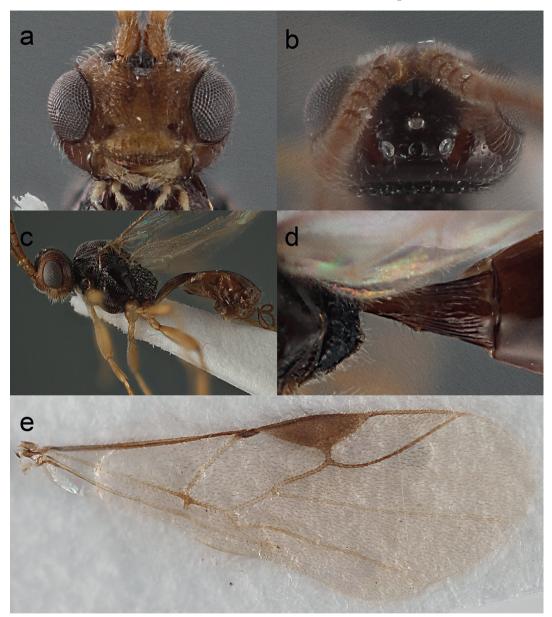


Figure 7. *Townesilitus deceptor*. – a) head frontal view, – b) head dorsal view, – c) habitus, – d) dorsal view of petiole, – e) fore wing. Figures a to d are specimen JS10_00233, figure e is from specimen JS10_00232.

Townesilitus deceptor. – a) ansikte, – b) huvud ovanifrån, – c) habitus, – d) petiolus ovanifrån – e) framvinge. Figurer a till d är individ JS10_00233, figur e är individ JS10_00232.

(3.2-1.6). Ocelli small with rather moderately deep pit between the posterior ocelli. Forewing length 1.8-2.2 mm (Fig. 6f). Mesoscutum on the middle lobe setate- punctate, side lobes only at their edges, otherwise smooth. Mesoscutum quite extensively punctate-reticulate, even on the smoother midsection with scattered few punctures (Fig. 6d). Propodeum laterally with strong carina framing the posterior part that is more or less hollow. Dorsally a strong pentagonal field, surrounding area almost smooth (Fig. 6e). Hind femur 5.3-5.6 times longer than wide (3.4.1-5.3). Length: 1.7-2.0 mm.

Diagnosis: *T. bicolor* has a relatively slender, paler coloured antennae with antennal segments 17-22.

Distribution: Western Palaearctic, Eastern Palaearctic. In Sweden *T. bicolor* has been found only in southern Sweden (Öland, Gotland, Småland and Skåne) (Fig. 5).

Ecology: *T. bicolor* is known to parasitize on *Psylloides attenuata* (Jolivet 1950), *Chaetocnema hortensis* and *C. aridula* (Pavlov 1960), Phyllotreta cruciferae, *P. striolata* in laboratory environment (Wylie & Loan 1984), *P. atra*, *P. undulata* and *Phyllotreta vittula* (Jolivet 1950).

Biology: Flight period in Sweden is from July to September, with some catches also at the end of June.

Discussion

Haeselbart (1988) noted three forms of this species and wrote as follow: 1) Small slender animals with 17-19 antennal segments. Ovipositor relatively long and with temples soon narrowed behind the eyes. 2) Most common form (Fig. 6b) – the form described in Haeselbarth 1988. Usually with 20-21 antennal segments and slightly larger and more robust. All reared on *Phyllotreta*, (Fig. 3) Larger specimens which are characterized by a short, slightly downwards bent ovipositor. The studied specimens from Sweden are mostly of form 2 as the description above tries to define. All three forms have been studied morphologically, unfortunately only one of the cryptic forms was successfully sequenced.

Townesilitus deceptor (Wesmael, 1835) *Microctonus deceptor* Wesmael, 1835

Redescription

Antennal segments: 22-28. Face slightly transversely wrinkled, shorthaired, forehead smooth and shiny (Fig. 7a). Maxillary palpi 6 segmented. Malar space less than half the width of mandible base. Eyes strongly converging. Head behind eyes rounded, slowly narrowed (Fig. 7b). Fore wing 2.5-3.2 mm (Fig. 7e). Mesoscutum moderately densely setate, on the sides somewhat sparse. Mesopleuron largely smooth and shiny. First metasomal tergite very slim, strongly broadened backward (Fig. 7c, d). Ovipositor weakly ventrally curved. Black brown. Length: 2.7-3.0.

Diagnosis: The largest of the *Townesilitus* species with an elongated marginal cell. Antennae broad and sturdy. A very variable species but wing venation, antennal shape and deep mesosternal sulcus is defining.

Distribution: Western Palaearctic, Eastern Palaearctic, Oriental. In Sweden *T. deceptor* has been found from Jämtland and southwards (Fig. 5).

Ecology: Noted hosts are *Altica deserticola* (Sergeev 2006), *A. quercetorum* (Tobias 1986).

Biology: Flight period in Sweden is from July to August. Some specimens were collected at the end of June and beginning of September.

Discussion

When studying the reference to the host *Plagio-seterna aenea* by Domenichini 1953 I noted that the description and illustrations of the parasitoid *Perilitus deceptor* cannot refer to *Townesilitus deceptor*. It is rather clear by the fact that Domenichini describes the petiolar sternite as almost membranous whilst on *Townesilitus* the sternite of the petiole is clearly sclerotized and fused.

Townesilitus fulviceps (Ruthe, 1856) Microctonus fulviceps Ruthe, 1856

Redescription

Antennal segments: 27-30. Face about twice as wide as high (Fig. 8a). Forehead smooth with a ridge that is lengthily striated between the an-

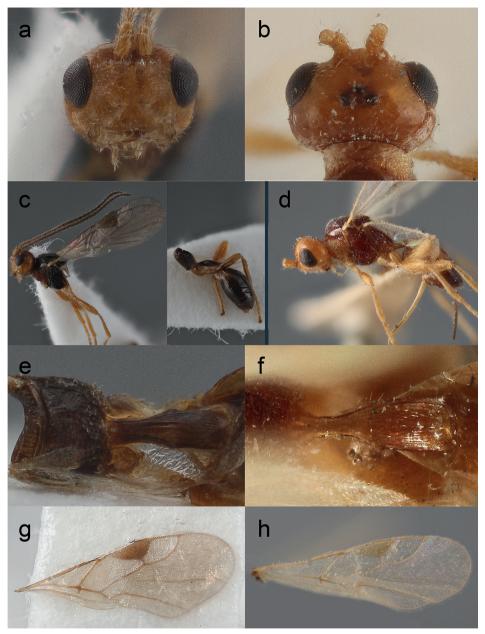


Figure 8. *Townesilitus fulviceps*: – a) head frontal view of specimen Euph_073, – b) head dorsal view of specimen Euph_073, – c) lateral view of mesonotum and metasoma of specimen Euph_073, – d) habitus view of type from BMNH, – e) dorsal view of petiole of specimen Euph_073, – f) dorsal view of petiole of type from BMNH, – g) fore wing of specimen Euph_073, – h) fore wing of type from BMNH.

Townesilitus fulviceps: – a) ansiktet på individ Euph_073, – b) huvudet ovanifrån av individ Euph_073, – c) mellan- och bakkropp från sidan på individ Euph_073, – d) habitus av typmaterialet från BMNH, – e) bild ovanifrån av individ Euph_073, – f) petiolus ovanifrån på typmaterialet från BMNH, – g) framvinge från individ Euph_073, – h) framvinge hos typmaterialet från BMNH.

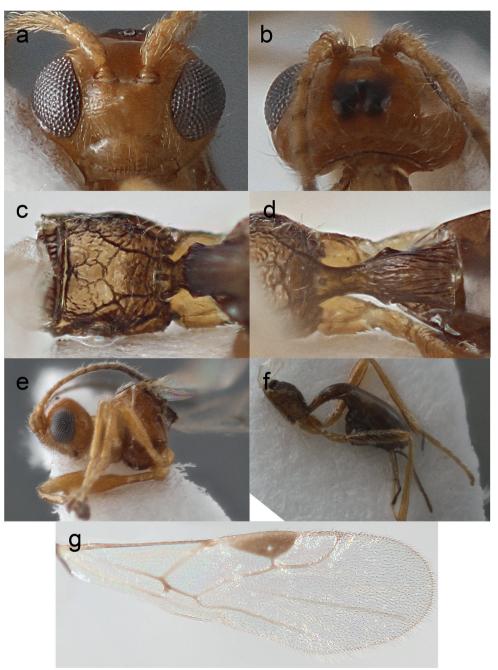


Figure 9. Townesilitus oelandicus sp. n.: -a) head frontal view, -b) head dorsal view, -c) dorsal view of propodeum, -d) dorsal view of petiole, -e) lateral view of mesonotum, -f) lateral view of metasoma, -g) fore wing. All images are of holotype Euph 138.

Townesilitus oelandicus sp. n.: – a) ansikte, – b) huvudet ovanifrån, – c) propodiet ovanifrån, – d) petiolus ovanifrån – e) framkropp från sidan, – f) bakkropp från sidan , – g) framvinge. Alla bilder visar holotypen Euph_138.

tennal bases. Malar space about half as large as the width of mandible base. Temples very wide, the head seen from above narrowed only behind the eyes (Fig. 8b). Fore wing 2.4-2.8 mm long (Fig. 8g, h). Mesoscutum punctured and setate, its sides at some places almost smooth. Metapleuron and propodeum everywhere densely reticulated-rugose, smoothly rounded with no clear distinction of a dorsal and posterior part (Fig. 8c, d). Petiole widened rapidly, usually before stigma, behind almost parallel sided, almost three times as long as the apical width and dorsally striated (Fig. 8e, f). Ovipositor wide and long, double bent. Sheaths longer than first tergite and only slightly shorter than the hind tibia. Length of type: 2.3 mm.

Diagnosis: A light-coloured species with small eyes. Well defined by the number of antennal segments, the head shape and shape of propodeum and ovipositor. A rare species but widespread. In Central Europe it is probably a heat loving species at lower elevations.

Distribution: Western Palaearctic, Eastern Palaearctic. In Sweden *T. fulviceps* has only been found in Skåne, southern Sweden (Fig. 5).

Biology: Flight period in Sweden in June. The specimen was sweep netted on 7th of June 2014.

Townesilitus oelandicus sp. n.

Description

Antennae with 17 segments, first and second flagellomere both 3.3 times as long as medially wide. Face 2.0 times as wide as high, with hardly visible weak striation almost smooth (Fig. 9a), covered with setae that are shorter (0.5 times) nearer the eyes. Eye protruding, glabrous, 1.3 times as wide as high. Lower tooth of mandible about 0.5 times length of upper tooth. Clypeus 0.95 times as wide as face, malar suture present but week, malar space 1.1 times width of mandible base. Ocelli minute, frons smooth. Temples behind eyes roundly narrowed, ocellar area dark brown (Fig. 9b). Mesopleuron anterior half with smooth rugosity, posterior half smooth (Fig. 9e). Mesoscutum smooth and with distinct notauli. Propodeum reticulate-rugose with two longitudinal carinae and dorsally a faint pentagonal area, the dorsal part of the propodeum 0.5 times

as long as the posterior sloping part, propodeum evenly rounded in shape (Fig. 9c, f). Petiole 2.3 times longer than apically wide, dorsally striated from about middle and onwards (Fig. 9d). Ovipositor sheets 0.65 mm. Hind femur 4.6 times longer than wide. Fore wing length 1.9 mm, stigma 2.2 times as long as marginal cell. Colour dark-yellow and body size 2.1 mm (Fig. 9g). The antenna basally yellow, darkening towards apex, from flagellomere 11 dark brown. Studied specimens: 1, DNA voucher Euph_138. Specimen length: 2.1 mm.

Diagnosis: Thin, slender and light yellow coloured, as some of the *T. bicolor* specimens in the analysis. The hind femur is sturdier than on *T. bicolor*.

Etymology: This species is named after the Swedish east coast island Öland, where Christer Hansson collected it in 2015.

Distribution: Found on Öland, Jortorpsåsen (Fig. 5). SWEDEN: Öland, Jordtorpsåsen, kärr, 56°40'35.0N, 16°33'30.8E, 27.vii.2015, C. Hansson

Biology: This species was caught by sweep net in 27th of July 2015. Host unknown.

Key to the Swedish (European) species of Townesilitus

- Hind femur (♀) 5.3-5.6 times longer than wide (♂ lectotype 4.2). Antennae 17-22 segments, majority between 18-19. Ovipositor S-shaped, slightly translucent and thin. Length of ovipositor sheets 0.6–0.7 mm. Length of fore wing (♀) 1.8-2.2 mm

Phylogenetic analysis

Based on molecular analysis of the barcode gene CO1 we can present here a phylogenetic tree over the species of *Townesilitus* occurring in Sweden (Fig. 10).

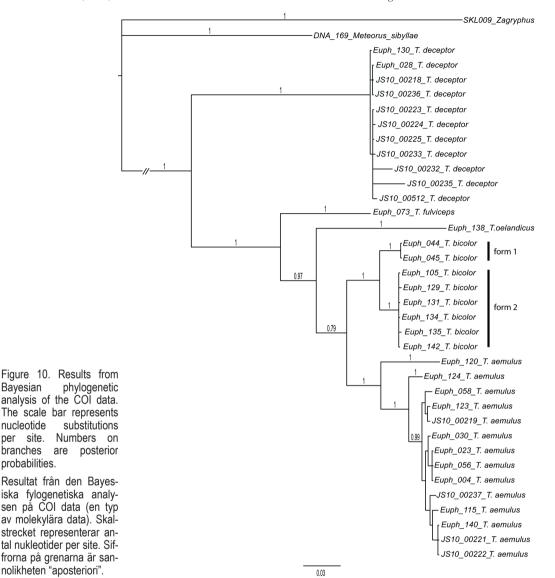
The Bayesian inference analysis show that the species fall into two well-supported clades. The first clade containing the species T. deceptor, sister to the clade that consists of the remaining four species. The species T. fulviceps and T. oelandicus are clearly displayed on their own branches, 17 % of the nucleotides differs between these two species. Though T. oelandicus lacks morphological characters to separate it from T. bicolor, this species is clearly differentiated by molecular characters. The nucleotide difference between T. oelandicus and the clade consisting of both T. bicolor and T. aemulus lies around 14-16%. T. bicolor is divided into two branches on their way to a species split but the lack of any morphological characters and the small difference (9 %) in nucleotide base pairs are not quite sufficient for such a split. The situation is the same for *T. aemulus*, voucher species Euph_120 vs. the clade containing the rest of *T. aemulus*.

Discussion

One of the main questions regarding T. bicolor and T. oelandicus is how to justify that the specimens identified as T. bicolor really are the true species T. bicolor, and why T. oelandicus is not. T. oelandicus is a cryptic species very similar to T. bicolor. The only morphological difference among the studied specimens vs. the type material is the size ratio of hind femur. Unfortunately there is only one specimen of T. oelandicus, a female, and the type material of *T. bicolor* are all males, and T. bicolor is very variable, as noted by Haeselbarth (1988). Therefore I assume that there is a higher probability to catch several specimens of T. bicolor rather than several specimens of some unknown species. I also think it is of value to note that T. bicolor may consist of several cryptic species and that I name one of them, based on molecular evidence. By doing so, future research might be able to add more phylogenetic and biological data on this species and finally find names for the rest of the cryptic species within T. bicolor. Unfortunately, I was only able to sequence two of the *T. bicolor* forms (Fig. 10), the other specimens of the studied material were not suitable for my current lab methods and I had no molecular results to present.

The phylogenetic results indicate that *Townesilitus* is divided into two clades. The first clade containing *T. deceptor* and the second clade containing *T. fulviceps*, *T. oelandicus*, *T. bicolor* and *T. aemulus* (Fig. 5). There are yet no good morphological characters and we have to rely on molecular characters to differentiate these species with confidence. Perhaps when more material of *T. bicolor* is collected some morphological characters might be discovered.

The apparent shortcomings of morphological characters are overcome by the value of DNA sequences and species names attached to the sequences presented here. To use both traditional morphology-based taxonomy and DNA based approaches would make future assessment on this group a bit easier.



Acknowledgements

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